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Multiple brain pathways and receptors underlying tobacco addiction

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ABSTRACT

Over the last 20 years much progress has been made in understanding the pharmacologic basis of tobacco addiction. In particular, the role of nicotine in reinforcing smoking behavior has been studied from a variety of perspectives. This article discusses two important aspects of this topic: (1) brain pathways underlying tobacco addiction; and (2) the actions of nicotine at nicotinic cholinergic receptors. Recent evidence will be reviewed indicating that nicotine reinforces smoking behavior by acting on more than one subtype of nicotinic receptor. Similarly, the role of several brain pathways in tobacco addiction will be considered. Tobacco addiction may thus be seen as a complex neuropsychopharmacological disorder; further progress in smoking cessation treatment may require that we address the multiple molecular and brain components of this addiction.

1. Introduction

Over the last 20 years much progress has been made in understanding the pharmacologic basis of tobacco addiction. Tobacco smoke contains thousands of constituents, including several known pharmacologically active compounds. These include nicotine and other alkaloids, such as nornicotine, anabasine and anatabine [1]. In addition, tobacco smoke contains substances such as harman and norharman that inhibit both A and B isoforms of the enzyme monoamine oxidase (MAO) [2].

Although other tobacco constituents besides nicotine may contribute to the addictive properties of cigarettes, research on tobacco addiction has primarily focused on the reinforcing effects of nicotine [3]. In addition to research establishing the link between nicotine reinforcement and tobacco addiction, there has been a great expansion in the range of pharmacotherapies available for smoking cessation; these include nicotine replacement therapy (NRT), bupropion and varenicline. Nonetheless, long-term success rates in smoking cessation treatment remain only about 20–25% at 1 year, even with the best currently available treatments [4]. It is likely that existing treatments fail to help the vast majority of smokers because they fall short of addressing the several distinct components of tobacco addiction. In the following discussion, these multiple components of addiction will be examined at two levels: (1) at the level of brain regions and pathways; and (2) at the receptor level. We will see that at both of these levels that multiple components – more than one brain region, and more than one receptor subtype – may underlie nicotine's addictive effects.

2. Multiple brain regions involved in tobacco dependence

This first main point of this article is that multiple brain regions are involved in nicotine reinforcement and tobacco

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addiction. Indeed, many brain regions have been implicated in both animal and human studies. Nicotine acts on brainstem, thalamic, striatal and cortical sites to enhance the release of several neurotransmitters, including dopamine, norepinephrine, acetylcholine, γ-aminobutyric acid (GABA), glutamate, and serotonin [5–8]. Nicotine has also been shown to modulate regional metabolic activity and cerebral blood flow [9–13]. The primary neural pathway that has been studied with respect to nicotine reinforcement has been the mesolimbic dopamine tract, with axons projecting from cell bodies in the ventral tegmental area (VTA) to frontal brain targets, including the nucleus accumbens. Nicotine stimulates burst firing in VTA neurons, resulting in enhanced dopamine release in the nucleus accumbens [7,14]. Activation of the dopamine reward pathway has been the hallmark of drugs of abuse, including cocaine, opiates, and alcohol [15].

However, human brain imaging studies suggest that additional brain regions and pathways are important in tobacco dependence. In a recent study [16], the involvement of specific brain regions in different aspects of tobacco dependence was studied using positron emission tomography (PET) to assess regional cerebral metabolic rate for glucose. In this study, smokers' degree of nicotine dependence was manipulated by switching to denicotinized cigarettes while wearing nicotine skin patches for 2 weeks. This manipulation reduced craving for cigarettes and nicotine dependence, as assessed with standard self-report measures. Three main findings emerged from this study. First, the manipulation of nicotine dependence significantly altered metabolic activity of the anterior cingulate cortex; this region had been previously implicated in studies of craving [17] as well as conflict and distress [18]. Second, changes in craving were inversely correlated with changes in metabolic activity of the striatum and orbitofrontal cortex, a target of midbrain dopamine pathways. A final, and perhaps most significant, finding of this study, was that changes in nicotine dependence scores were positively correlated with changes in metabolic activity of the thalamus. The thalamus is well known for its role in gating afferent and efferent stimulation to and from the cerebral cortex; importantly, the thalamus is also a region that contains a very high density of nicotinic receptors [19]. Metabolic activity of the thalamus – and of the amygdala – also showed strong correlations with reported smoking for calming effects. Given that one of the most frequently cited reasons for smoking is to calm down under stress, the thalamus and amygdala may play a greater role in tobacco addiction than generally recognized.

In addition to these brain regions, the insula has been suggested to be important in tobacco dependence. A recent study found that smokers who suffered stroke damage to the insula reported quitting smoking with little difficulty relative to smokers with lesions in other brain regions [20]. The insula is important for processing interoceptive cues, and the relative ease of quitting in the insula-lesioned patients could have been a result of the absence of interoceptive cues associated with tobacco withdrawal, or the absence of smoking-related internal sensations that are perceived as rewarding.

Aside from the brain regions just described, several cortical pathways have also been implicated in studies of cue-elicited craving for cigarettes. For example, visual cues or scripts that elicit craving not only activate subcortical regions, such as striatum, thalamus and amygdala, but also cortical regions such as anterior cingulate cortex (mentioned above), prefrontal cortex, temporal cortex, occipital cortex, insula, precuneus and cuneus [21–23].

Yet another brain region that may turn out to play a role in tobacco addiction is the habenula. The habenula is a suprathalamic structure that projects *via* the fasciculus retroflexus pathway to several important brain regions, including the interpeduncular nucleus and VTA [24]. The habenula is also connected to the cholinergic neurons of the basal forebrain, and thus is well situated to coordinate cholinergic transmission in the brain [25].

The medial portion of the habenula is rich in several subtypes of nicotinic receptors [26,27]. The medial habenula projects to the lateral habenula which in turn exerts a strong inhibitory tone on midbrain dopamine neurons. Midbrain dopamine neurons in turn project back to the medial habenula [28]. These reciprocal pathways between the habenula and VTA are consistent with the role of the habenula in reinforcement or reward. Intriguingly, the habenular response to signals of nonreward is attenuated among individuals with schizophrenia [29,30]. Although speculative, the inordinately high prevalence of smoking in the schizophrenic population might be linked to the deficit in habenular function. It is also interesting that the habenula and associated fasciculus retroflexus are the only brain structures to show prominent degeneration following chronic exposure to nicotine [31]. This degeneration was observed in rats at doses as low as 5 mg/(kg day), yielding plasma concentrations of approximately 50 ng/ml, within the range to which human smokers are exposed. The habenula has also been implicated in stress responses [32], which are relevant to cigarette smoking because stress is known to be a key trigger of smoking relapse [33]. It is conceivable that nicotine modulates stress responses in part by acting on the habenula, and this could contribute to tobacco addiction.

3. Multiple nicotinic receptors underlying tobacco dependence

The effects of nicotine at the level of brain regions and pathways result from nicotine acting on the molecular level at nicotinic receptors. This leads to the second main point: more than one nicotinic receptor subtype is involved in tobacco addiction.

Nicotinic receptors at the neuromuscular junction were discovered over 100 years ago [34], but what appeared initially to be a single type of receptor was found in the 1950s to consist of two general categories: neuromuscular junction and autonomic ganglia. Subsequently, nicotinic receptors were discovered in the central nervous system (CNS). A great deal of light has been shed on the mechanisms of action and functional significance of nicotinic receptors in the CNS [6,35–37]. Nicotinic receptors subserve a wide range of inter-related functions in the brain, including memory and attention, stress responses, and reinforcement [38–40]. Again, although initially seeming to fall into a simple dichotomomy of "low-affinity" and "high-affinity" receptors, based on binding affinity for nicotine [41],

it was subsequently discovered that there is a multiplicity of nicotinic receptor subtypes. Neuronal nicotinic receptor subtypes in mammals consist of different combinations of eight alpha subunits (α2–α7, α9–α10) and three beta subunits (β2–β4), which are assembled into pentameric, ligand-gated ion channels [35,36]. Although many subunit combinations are possible, rules of receptor assembly restrict the diversity of subtypes expressed [37,42].

When nicotine binds to a receptor, it may activate that receptor, resulting in the ion-channel opening transiently. Subsequently, the receptor will become desensitized (closed-channel conformation); later, after nicotine dissociates from the receptor, it will recover from desensitization and can be activated again. This dynamic process of activation, desensitization, and re-sensitization, is further complicated by the possible mobilization nicotinic receptors from internal pools [43].

Different nicotinic receptor subtypes will show different probabilities of activation or desensitization in response to varying concentrations of nicotine. In order for a receptor subtype plausibly to play a role in cigarette smoking reinforcement, it must be responsive – either in terms of activation or desensitization – to the changing concentrations of nicotine resulting from smoking a cigarette.

Smoking one cigarette increases the concentration of nicotine in arterial blood reaching the brain by about 20–70 ng/ml (100–400 nM) [44–46]. Before smoking, the ''trough'' concentration of nicotine, measured in venous blood, is as low as 4 ng/ml (25 nM), before the first cigarette of the day, and reaches a steady-state level of about 30 ng/ml (185 nM) by the afternoon [47]. Assuming that nicotine crosses the blood-brain barrier by simple passive diffusion, the concentration of free nicotine in brain (available for interaction with receptors) cannot be higher than the peak concentration of nicotine in arterial blood. Nor can the free concentration in brain be smaller than the nicotine concentration in venous blood. Therefore, the brain concentration of free nicotine can fluctuate as much as from about 4 ng/ml (25 nM) to 74 ng/ml (460 nM) after the first morning cigarette, and from about 30 ng/ml (185 nM) to 100 ng/ml (620 nM) during afternoon smoking. Because the washout of nicotine from the brain is slower than that from the blood, the difference in brain free nicotine concentration between pre-smoking and post-smoking conditions could be even smaller than the fluctuations measured in blood.

Efforts have been made to link tobacco addiction to the action of nicotine at one or more specific nicotinic receptor subtypes. In this regard, most of the attention has been focused on α4β2 receptors that bind nicotine with high affinity. These receptors comprise the majority of nicotinic receptor binding sites in the brain, and are found in many regions, including brainstem, midbrain, thalamus, habenula, striatum and cortex. They are believed to be involved in reinforcement and dependence [48], as well as in nicotine-evoked dopamine release [49]. One important line of evidence implicating α4β2 receptors in nicotine reinforcement derives from genetic ''knock-out'' and ''knock-in'' gain-of-function mutant studies in mice [50–52]. Several strains of mice have been bred that lack a specific nicotinic receptor subunit gene, and have been studied in terms of their behavioral phenotypes. A key finding is that, in contrast to wild-type controls, β2 knock-out mice do not self-administer nicotine [53]. On the other hand, mice with a gain-of-function mutation that potentiates α4-subunit activity, show exaggerated signs of nicotine dependence—including reward, tolerance and sensitization [54]. Taken together with the results from β2 knock-out studies, it appears that α4β2 receptors are important for nicotine self-administration.

However, the actions of nicotine at high-affinity α4β2 receptors are not sufficient to explain the rewarding effects of cigarettes. The reason is that the nicotine concentrations present throughout the day are outside of the range in which these receptors are sensitive, in terms of responding with greater activation or desensitization to the changes in nicotine concentrations after cigarette smoking. That is, a typical smoker will smoke cigarettes at roughly 30–90 min intervals throughout the day; yet the first 1–2 cigarettes of the day almost completely saturate high-affinity α4β2 receptor-binding sites. The saturating effect of low doses of nicotine have been demonstrated in a study using the radiotracer 2-[18F]fluoro-3-(2(S)-azetidinylmethoxy) pyridine (abbreviated 2-FA), which binds with high affinity to α4β2 nicotinic receptors. The displacement of 2-FA by nicotine inhaled in cigarette smoke was measured using PET [55]. Inhalation of only 1 or 2 puffs of nicotine-containing smoke occupied approximately 50% of α4β2 nicotinic acetylcholine receptor-binding sites in the brain. Smoking an entire cigarette resulted in approximately 90% occupancy of binding sites for several hours. The equilibrium binding constant was calculated to be only 0.87 ng/ml (5.4 nM). Thus, at typical concentrations of nicotine, most high-affinity α4β2 receptor-binding sites will be occupied; further, many α4β2 receptors will be in a desensitized, rather than active state [56].

Thus, if only α4β2 receptors mediated the reinforcing effects of nicotine, it is puzzling that smokers continue to smoke at regular intervals throughout the entire day, because there should be little additional effect of smoking a cigarette – either to activate α4β2 receptors or to induce further receptor desensitization – if previous cigarettes have produced essentially full receptor occupancy. The puzzle is resolved if one postulates that there is one or more additional subtype of nicotinic receptor that remains available for nicotine actions throughout the day. Nicotinic receptors with lower affinity will not be susceptible to saturation by nicotine, and can continue to respond to cigarettes throughout the day.

It could be argued that another possible resolution to the puzzle of maintained smoking throughout the day is that continued smoking is sustained by conditioned reinforcement from smoking-related sensory cues [38] rather than primary reinforcement from nicotine. Indeed, a large body of research has implicated sensory cues and/or non-nicotine smoke constituents in cigarette addiction [57]. For example, attenuation of both nicotine-related and non-nicotine-related airway sensations reduces smoking satisfaction [58,59]. Moreover, craving as well as other tobacco withdrawal symptoms are alleviated by smoking denicotinized cigarettes [60,61]. Studies of nicotine self-administration in rats have also demonstrated a potent effect of sensory cues; nicotine administration amplifies the effect of these cues [62]. Thus, it might appear plausible to speculate that even if nicotine has little direct

effect after the first 1–2 cigarettes of the morning, sensorimotor cues could provide sufficient reinforcement to maintain smoking behavior for the rest of the day.

However, conditioned cues or other non-nicotine factors cannot fully explain the observation that dose-related pharmacologic effects of nicotine could be observed throughout the day. For example, in studies using nicotine skin patches (which are relatively devoid of sensory cues and other non-nicotine factors), administration of incremental doses ranging from 7 mg/24 h to 63 mg/24 h produces a stepwise suppression of withdrawal symptoms and smoking behavior [63–68]. Given that the levels of nicotine achieved by even a 14 mg/24 h patch should occupy over 90% of high-affinity α4β2 receptor-binding sites, the added effect of higher dose patches suggests that some other receptor subtype comes into play.

An additional, and related, line of evidence supports the view that high-affinity binding to α4β2 receptors is not sufficient to explain nicotine's actions. Clinical trials have shown that the nicotinic receptor antagonist mecamylamine significantly increases rates of smoking cessation when added to 21 mg/24 h nicotine patch treatment [57,69,70]. The additional therapeutic effects of mecamylamine, over and above the effects of a 21 mg nicotine patch – which as noted previously would be expected to saturate high-affinity α4β2 receptor-binding sites – points to the potential involvement of some other receptor subtype. As will be discussed below, mecamylamine is highly potent at blocking nicotinic receptors other than the α4β2 subtype.

If high-affinity binding to α4β2 receptors cannot completely explain nicotine's effects, then what other mechanisms might be involved? One mechanism would be lower affinity binding sites for nicotine, which would retain their sensitivity to nicotine throughout the day. Indeed, it has been shown that there are low-affinity binding sites associated with α4β2 receptors [37]. Moreover, the sensitivity of these receptors may be affected by their stoichiometry (number of alpha and beta subunits) [71,72].

Another possible subtype to consider is the homomeric α7 receptor (consisting of five alpha 7 subunits), which is found predominantly in the hippocampus, but is also present in other brain regions (e.g., midbrain, thalamus and cortex). These receptors are thought to mediate some of the cognitive-enhancing and sensory-gating effects of nicotine [73–75]. Moreover, α7 receptors may contribute to nicotine reinforcement by modulating dopamine release *via* acting on glutamatergic terminals that in turn synapse on dopamine neurons [49,76,77]. Alpha-7 receptors also have a broader neuromodulatory role arising from their influence on calcium influx [78].

Recent research with genetic knock-out mice lacking the α7 subunit also supports a role in nicotine self-administration. Although α7 knock-out mice show no difference from wild-type controls in their initial preference for nicotine [48], their nicotine self-administration behavior declines over several weeks [79]. These results support a potential role for α7-subunit containing receptors in nicotine dependence.

One difficulty with accepting a role for α7 nicotinic receptors in addiction has been that in some *Xenopus* oocyte expression studies, these receptors show a very high threshold for activation, with an EC50 value of approximately 15,000 ng/ml (100 μM) [80], much higher than peak nicotine concentrations reaching the brain. Based on these results, smoking a cigarette would not be expected to activate more than a small fraction (<1%) of α7 receptors. However, it has been suggested that receptor desensitization complicates the analysis of concentration–response relationships for α7 receptors, and that the actual EC50 value in the oocyte model is much lower [81]. Moreover, studies of native α7-subunit containing receptors have demonstrated effects on presynaptic release on neurotransmitter at relatively low nicotine concentrations, e.g.15 ng/ml (100 nM), well within the range to which smokers are exposed [82]. Possibly these native receptors are heteromeric, having a different subunit composition than the homomeric α7 receptors studied in oocytes. Alternatively, other differences between the micro-environment of the nerve terminal as compared with the oocyte might affect sensitivity to nicotine.

Yet another possibility is that desensitization, rather than activation, of α7 receptors, is reinforcing for smokers. However, reinforcement would likely to depend on an enhancement in dopamine release, or an enhancement in cognitive function, both of which result from α7-receptor activation. This is shown by the observation that these effects are blocked by the α7-antagonist methyllycaconitine (MLA) [77,83]. Desensitization would be expected to turn receptors "off" and resemble the effect of an antagonist, which would not be expected to be reinforcing.

In addition to α7 receptors, receptors containing the α6 subunit may be important in nicotine addiction. These receptors, which may also incorporate β2, β3 and other subunits, are found within catecholaminergic nuclei, including the locus coeruleus and VTA [84–88]; the VTA has been shown to be a primary site for nicotine reinforcement [7]. Alpha-6 subunit containing receptors have indeed been shown to facilitate dopamine release from nerve terminals, but the importance of this effect for nicotine reinforcement has been questioned, in that non-α6 subunit containing receptors on the dopamine cell bodies seem to be more critical for nicotine-evoked dopamine release and for nicotine self-administration [89].

Besides the above nicotinic receptor subtypes, receptors containing the β4 subunit could play an important role in tobacco addiction. Receptors incorporating the β4 subunit include the α3β4 or "ganglionic" subtype (also incorporating α5, β2 and perhaps other subunits), which affect neurotransmission in the autonomic nervous system, and are also found in discrete brain regions including the habenula, locus coeruleus and interpeduncular nucleus [90–92]. Another potential β4-subunit containing receptor is the α4β4 receptor. Although they have not been detected in mammalian brain, α4β4 receptors have been shown to be functional when expressed in *Xenopus* oocytes [80] and human cell lines [93].

Receptors containing the β4 subunit have lower binding affinity than α4β2 receptors and do not become saturated at typical "trough" levels of nicotine found in smokers' plasma [80,94,95]. They are also less prone to rapid desensitization than are α4β2 receptors [56,80,94]. Thus, based on considerations of receptor occupancy and resistance to desensitization, β4-subunit containing receptors could plausibly mediate the reinforcing effects of nicotine throughout the day.

Further support for the plausible role of β4-subunit containing receptors in nicotine dependence comes from studies evaluating mecamylamine in smoking cessation treatment. As mentioned above, several studies have shown efficacy for mecamylamine. Unlike an agonist, which can produce physiological effects by activating a small fraction of receptors, an antagonist such as mecamylamine must block a substantial fraction of receptors to significantly alter the response to an agonist such as nicotine. In order to have these behavioral effects at the 5–10 mg daily doses used in these studies, which yield plasma levels of approximately 16 ng/ml (0.1 μM), mecamylamine must be very potent at blocking the relevant subtype(s) of nicotinic receptors. Although widely considered a nonspecific open-channel blocker, mecamylamine in fact has different potencies in blocking different nicotinic receptor subtypes. For example, based on oocyte expression studies, mecamylamine has been found to be relatively weak in terms of blocking α7 receptors (IC50 of 1.6–6.9 μM), and is only modestly effective at blocking α4β2 receptors (IC50 of 0.6–2.5 μM); however, it potently blocks ganglionic-type α3β4 receptors (IC50 of 0.09–0.64 μM) [96] as well as α4β4 receptors [80]. The observed side effect of constipation in clinical trials confirms that 5–10 mg/day mecamylamine produces sufficient concentrations to block ganglionic nicotinic receptors. This receptor subtype, or other β4-subunit containing nicotinic receptors, would be plausible candidates for mediating the therapeutic actions of mecamylamine.

Additional evidence for the role of β4-subunit containing receptors has been found in genetic knock-out studies. As mentioned previously, β2 knock-out mice do not self-administer nicotine to the same extent as controls [53]; nevertheless, after chronic nicotine administration, β2 knock-out mice still show signs of dependence: mecamylamine injections precipitate behavioral signs of withdrawal in these animals [97]. In contrast, in a similar paradigm, β4 knock-out animals show little or no behavioral signs of withdrawal [98]. This result further supports a role for β4-containing receptors in nicotine dependence.

In summary, nicotinic receptors other than the α4β2 subtype are likely to play a significant role in nicotine reinforcement and tobacco addiction. This role may involve some of the known subtypes of receptors which contain α6, α7 or β4-subunits, or may involve additional novel nicotinic subunit combinations yet to be fully characterized.

4. Conclusion

To summarize, tobacco addiction arises in part from the reinforcing actions of nicotine at nicotinic receptors. Although much research attention has focused on the prevalent α4β2 receptor subtype, additional nicotinic receptor subtypes are likely involved in both the reinforcing effects of nicotine, and in the therapeutic effects of smoking cessation treatments such as nicotine replacement or nicotinic receptor blockade.

Actions of nicotine at the receptor level in turn modulate the activity of several brain regions and interconnecting pathways that subserve distinct functions. These may include: cortical pathways responsible for sensory processing and executive functions; striatal pathways mediating reward-approach behavior; insula processing of interoceptive sensations; thalamus and amygdala, mediating evaluation and gating of sensory cues; and potentially the habenula, with its high density of nicotinic receptors and broad functions modulating reward and stress responses. In order to develop truly effective smoking cessation therapies, it may be necessary to address these multiple components of tobacco addiction.

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